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Evidence, from Combined Segregation and Linkage Analysis, That a Variant of the Angiotensin I–converting Enzyme (ACE) Gene Controls Plasma ACE Levels

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Summary

The hypothesis of a genetic control of plasma angiotensin I–converting enzyme (ACE) level has been suggested both by segregation analysis and by the identification of an insertion/deletion (I/D) polymorphism of the ACE gene, a polymorphism contributing much to the variability of ACE level. To elucidate whether the I/D polymorphism was directly involved in the genetic regulation, plasma ACE activity and genotype for the I/D polymorphism were both measured in a sample of 98 healthy nuclear families. The pattern of familial correlations of ACE level was compatible with a zero correlation between spouses and equal parent-offspring and sib-sib correlations (.24 ± .04). A segregation analysis indicated that this familial resemblance could be entirely explained by the transmission of a codominant major gene. The I/D polymorphism was associated with marked differences of ACE levels, although these differences were less pronounced than those observed in the segregation analysis. After adjustment for the polymorphism effects, the residual heritability (.280 ± .096) was significant. Finally, a combined segregation and linkage analysis provided evidence that the major-gene effect was due to a variant of the ACE gene, in strong linkage disequilibrium with the I/D polymorphism. The marker allele I appeared always associated with the major-gene allele s characterized by lower ACE levels. The frequency of allele I was .431 ± .025, and that of major allele s was .557 ± .041. The major gene had codominant effects equal to 1.3 residual SDs and accounted for 44% of the total variability of ACE level, as compared with 28% for the I/D polymorphism. The I/D polymorphism should constitute a powerful tool for identifying the ACE gene variant involved in the regulation of ACE level.

Introduction

Angiotensin I–converting enzyme (ACE) plays an important role in circulatory homeostasis, by catalyzing the conversion of angiotensin I to angiotensin II, a potent vasopressor, and by inactivating bradykinin, a vasodilator (Erdös and Skidgel 1987). ACE is present as a membrane-bound enzyme in endothelial cells, as well as in different types of epithelial cells. A circulating form of ACE is found in plasma, which is most likely released by the vascular endothelial cells (Erdös and Skidgel 1987). Abnormal levels of plasma ACE have been observed in the course of several granulomatous diseases, and this abnormality is widely used for the diagnosis and follow-up of sarcoidosis (Lieberman 1975).

In healthy individuals, plasma ACE levels remain highly stable when measured repeatedly in a given subject (Dux et al. 1984), whereas there is a large interindividual variability, since ACE levels can differ up to fivefold among subjects (Alhenc-Gelas et al. 1991). A search for environmental or hormonal parameters accounting for this interindividual variability has revealed only weak associations (Alhenc-Gelas et al.

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1991). On the other hand, a genetic control of circulating ACE was strongly suggested by the results of a family study showing that familial similarity in ACE levels was compatible with the transmission of a major gene, explaining a large part of the interindividual variability of the enzyme level (Cambien et al. 1988). This study was followed by the identification of an insertion/deletion (I/D) polymorphism of the ACE gene, a polymorphism that was associated with marked differences of serum ACE levels in unrelated healthy individuals (Rigat et al. 1990).

In order to elucidate whether the I/D polymorphism was directly involved in the genetic control of ACE level or was only a marker in linkage disequilibrium with a regulatory variant of the ACE gene, a new family study was carried out. We report here the results of a combined segregation and linkage analysis carried in this family study.

Subjects and Methods

Family Data

The families were recruited in the Center for Preventive Medicine in Vandoeuvre-lès-Nancy, France. The population screened in this center is made up of families living in the Nancy area and volunteering to have a free health-checkup examination (Deschamps 1987). During 1989–90, 98 nuclear families of Caucasian origin, composed of both parents age <60 years and at least two offspring age ≥9 years, agreed to participate in a study on familial hypertension risk factors. The sample comprised 404 subjects, including 208 offspring (average number of offspring 2.1). Exclusion criteria were presence of acute or chronic disease, body mass index >28 kg/m^2, antihypertensive-drug intake, alcohol consumption >50 g/24 h, and gamma-glutamyl-transferase >50 IU/liter.

Blood pressure was measured by an automatic device (Dinamap; Critikon), with the subject in the recumbent position. Seven measurements were taken at 3-min intervals, and the same procedure was repeated at a second visit 15 d later. Analysis was performed on the mean of all 14 measurements.

Plasma ACE Activity Measurement and DNA Extraction

The enzymatic activity of plasma ACE was measured on a synthetic substrate (FAPGG) according to a method described elsewhere (Beneteau et al. 1986). Genomic DNA from subjects was prepared by standard techniques (Marcadet et al. 1986).

Detection of the Insertion/Deletion Polymorphism of the ACE Gene

The I/D polymorphism detected by the ACE cDNA probe (Rigat et al. 1990) was localized to intron 16 of the ACE gene (Hubert et al. 1991), by restriction-endonuclease mapping using short ACE cDNA fragments and by size analyses of amplified introns from individuals bearing the insertion or the deletion genotype. Nucleotide sequence of the insertion and of the flanking regions was determined by the chain-termination method (Sanger) on a cloned genomic fragment of the ACE gene, pHA4.3 (Hubert et al. 1991). The insertion itself corresponds to an *alu* repetitive sequence and is 287 bp long. Two oligonucleotides, SC1407, 5′ CTGGAGACCACTCCCATCCTTTCT 3′; and BR2352, 5′ GATGTGGCCATCACATTCGTCAGAT 3′, were designed to amplify the polymorphic fragment from human genomic DNA, and they allow the detection of a 190-bp fragment in the absence of the insertion and of a 490-bp fragment in the presence of the insertion. Amplification reactions were carried out in standard conditions (Saiki et al. 1988), in 3 mM $MgCl_2$, using *Taq* polymerase (Cetus) and a PTC-100 thermal cycler (MJ Research). The DNA was amplified for 30 cycles, each with denaturation at 94°C for 1 min, annealing at 58°C for 1 min, and extension at 72°C for 2 min. Reaction products were analyzed on agarose gel, for allele identification.

Statistical Methods

Analysis of variance. — The association of the I/D polymorphism with ACE and blood pressure levels was tested by a one-way analysis of variance, separately in parents and offspring. The difference of allele effects between parents and offspring was investigated by testing the genotype × generation term in a two-way analysis of variance. This method was applied as a first approach to investigate the effects of the I/D polymorphism, although individuals within families could not be considered as independent.

Computation of familial correlations. — Familial correlations were computed jointly by using the maximum-likelihood method proposed by Donner and Koval (1981). The distribution of the trait is assumed to be multivariate normal within each family, with parameters identical whatever the family size.

Combined segregation and linkage analysis. — Combined segregation and linkage analysis can be viewed as an extension of segregation analysis applied to a multivariate phenotype composed of one trait and one (or several) marker(s) (Bonney et al. 1988). In segregation

analysis, the likelihood function is written as $L(x) = \Sigma_g P(x/g)P(g)$, where x is the phenotype determined by the trait, g is the unobserved genotype underlying the trait, $P(x/g)$ is the conditional probability of x given g (penetrance function), and $P(g)$ is the prior probability of the genotype. The generalization of this function to a multivariate phenotype including one trait and q linked markers gives $L(x,m) = \Sigma_g P(x,m/g)P(g)$, where m is the q vector of marker phenotypes, and g now spans all the loci underlying the trait and the markers. The term $P(x,m/g)$ can be factored as $P(x/g) \times P(m/x,g)$. The second factor is unity because m is completely determined, given the genotype. The first factor is the penetrance function conditional on the genotype, which only depends on the major genotype underlying the trait. Thus, the only part of the likelihood which differs from the univariate segregation analysis concerns the prior probabilities of genotypes. In segregation analysis, these probabilities are generally taken to be in Hardy-Weinberg proportions for parents and are derived from transmission probabilities for offspring. In combined segregation and linkage analysis, the probabilities of genotypes are modified by the linkage relationship and the segregation of genotypes at the marker loci. The genotypic probabilities are functions of allele frequencies at the different loci, recombination rate, and gametic association between loci. In this context, the "univariate" segregation analysis corresponds to the particular case where (*a*) the major locus underlying the trait is unlinked to the marker loci and (*b*) there is no gametic association between them.

For the present application, the data consisted of a quantitative trait, i.e., the ACE level, and a two-allele marker, i.e., the I/D polymorphism. Since the polymorphism is situated within the ACE gene, a zero recombination rate was assumed between the marker locus and the major locus. The letters "s" and "S" were used to denote the two alleles of the major gene underlying the trait, and the genotype probabilities were parametrized in terms of f_I, i.e., the frequency of marker allele I, and π_I and π_D, i.e., the frequencies of allele s conditional to allele I and allele D of the marker, respectively. Note that the major-gene frequency, q_s, is no more a parameter of the model, since it is completely determined by the relation $q_s = f_I\pi_I + (1 - f_I)\pi_D$.

Various genetic hypotheses can be tested on these parameters. Equality of π_I and π_D means gametic equilibrium, whereas a significant difference indicates an association between the loci. Fixing π_I or π_D to 1 or 0 provides a test for complete gametic disequilibrium. A model specifying $\pi_I = 1$ and $\pi_D = 0$ indicates a complete identity between the two loci. This particular model also allows one to estimate—and test the significance of—the effects of the marker locus on the quantitative phenotype. This refers to the measured genotype analysis described by Boerwinkle et al. (1986). Finally, a model with the restrictions $\pi_I = 1$ and $\pi_D = 1$ corresponds to the absence of major gene.

Within each major genotype, the penetrance function was that of the mixed model (Elston and Stewart 1971; Morton and MacLean 1974; Lalouel et al. 1983). The parameters are the three genotype-specific means μ_{ss}, μ_{Ss}, and μ_{SS}; the residual variance σ^2 (after adjustment for the major-gene effect), which is common to all genotypes; and the heritability h^2, which is the proportion of the residual variance attributable to a polygenic (or, more generally, multifactorial transmissible) component.

The analysis was performed by using the Pedigree Analysis Package (PAP) program (Hasstedt 1989). We performed successively (1) a measured genotype analysis to examine the contribution of the I/D polymorphism to the variability of the ACE level, (2) a segregation analysis to investigate the possible role of an unmeasured major gene, and (3), finally, a combined segregation and linkage analysis to test the hypothesis of linkage disequilibrium between the I/D marker and the major gene. In measured genotype analysis and combined segregation and linkage analysis, Mendelian segregation was assumed, whereas in segregation analysis this hypothesis was tested by comparing the Mendelian and the environmental models against the model of general transmission (Demenais et al. 1986). All hypotheses were tested by means of the likelihood-ratio criterion.

In offspring, the ACE level appeared negatively correlated with age, and an adjustment prior to analysis was made on age and age^2, separately for sons and daughters. In parents, the ACE level did not vary with age, and no adjustment was made. Since the mixed model implemented in PAP assumes equality of variances between parents and offspring, the ACE level was standardized separately in parents and offspring. The working variable for all familial analyses had, then, a zero mean and a variance equal to unity.

Results

The means and SDs of age, systolic and diastolic blood pressures, and ACE level are given in table 1.

Table 1

Mean (SD) Values for Age, Blood Pressure, and Plasma ACE Level

	Fathers (n = 98)	Mothers (n = 98)	Sons (n = 122)	Daughters (n = 86)
Age (in years)	41.4 (4.1)	39.4 (3.4)	14.4 (2.9)	14.7 (3.0)
Systolic blood pressure (in mm Hg)	125.5 (10.4)	115.6 (11.6)	116.3 (9.7)	113.8 (7.2)
Diastolic blood pressure (in mm Hg)	76.6 (7.7)	71.2 (8.1)	61.3 (5.5)	62.9 (5.7)
Plasma ACE (in IU/liter)	89.7 (29.5)	84.7 (27.2)	128.3 (45.5)	106.7 (39.0)

ACE means were significantly higher in offspring than in parents ($P < .001$) and in sons than in daughters ($P < .001$). Age and age^2 explained 8% and 13% of ACE variance in sons and daughters, respectively. The skewness of the ACE distribution was not significant for any group of relatives. Correlation between ACE level and blood pressure was not significant in parents. In offspring, after adjustment for age, there was a significant correlation of ACE level with systolic (r = .19; P = .006) and diastolic blood pressure (r = .13; P = .05).

The I/D polymorphism was in Hardy-Weinberg equilibrium. By one-way analysis of variance, marked differences of ACE levels were observed between the three I/D genotype classes in parents and in offspring (table 2). The mean effect of allele D appeared higher in offspring than in parents, but this difference was not statistically significant. No association was found between this polymorphism and blood pressure (table 2).

Familial Correlations of ACE Level

Familial similarity in ACE level was highly significant (χ^2 = 30.56 with 4 df; $P < .001$). The pattern of familial correlations was compatible with a zero correlation between spouses and with equal correlations (r = .24 ± .04) among various pairs of biological relatives (χ^2 = 1.72 with 3 df; not significant [NS]).

Measured Genotype Analysis

The results of measured genotype analysis are shown in table 3. The model including effects of the I/D polymorphism (model 1) was much better supported by the data than was the model without effect (model 0) (χ^2 = 141.33 with 3 df; $P < .001$). Residual polygenic effects were also significant, as indicated by the comparison of model 2 with model 1 (χ^2 = 9.39 with 1 df; $P < .001$). Finally, the best-fitting model indicated an allele frequency of .431 ± .025 and codominant effects (μ_{II} = −0.931 ± 0.105; μ_{ID} = −0.087 ± 0.065; and μ_{DD} = 0.657 ± 0.081). The residual h^2 was estimated as .280 ± .096, and the residual SD was estimated as .850 ± .031. The marker contributed for 28% of the total variance of ACE level.

Segregation Analysis

The results of segregation analysis are shown in table 4. The mixed model assuming a codominant major gene and a residual polygenic component (model 1) was better supported than was the model without gene

Table 2

Mean (SD) Values of Blood Pressure and Plasma ACE Level, by I/D Genotype

	Genotype II	Genotype ID	Genotype DD	P Value
Parents:	n = 33	n = 103	n = 60	
SBP (in mm Hg)	119.1 (11.0)	121.3 (12.5)	120.0 (11.9)	NS
DBP (in mm Hg)	71.9 (6.7)	74.7 (8.9)	73.5 (8.1)	NS
ACE level (in IU/liter)	61.1 (23.0)	83.7 (23.8)	107.5 (24.0)	<.001
Offspring:	n = 43	n = 94	n = 71	
SBP (in mm Hg)	113.7 (9.5)	115.5 (8.7)	115.8 (8.5)	NS
DBP (in mm Hg)	61.1 (5.6)	61.6 (5.8)	63.0 (5.3)	NS
ACE level (in IU/liter)	81.7 (28.4)	119.9 (38.0)	141.4 (44.7)	<.001

Table 3

Measured Genotype Analysis: Hypotheses Testing Contribution of I/D Polymorphism to Variability of Plasma ACE Level

	Model 1	Model 2
Frequency of allele I: f_I	.431	.431
Genotype-specific means:		
μ_{II}	−.930	−.931
μ_{ID}	−.079	−.087
μ_{DD}	.650	.657
Residual SD	.850	.850
Residual h^2	(0)[a]	.280
Alternate model	0[b]	1
χ^2	141.33***	9.39**
df	3	1

[a] Parameter was fixed at value shown.
[b] Model 0 is without polymorphism effects.
** $P < .01$.
*** $P < .001$.

effects (model 0) (χ^2 = 37.27 with 4 df; $P < .001$). In this model, the parameter of h^2 converged to 0, indicating an absence of residual polygenic effects. Mendelian segregation was not rejected when compared with general transmission (model 2) (χ^2 = 6.18 with 3 df; NS), whereas the absence of transmission (model 3) was rejected (χ^2 = 34.70 with 2 df; $P <$ .001). The best-fitting model indicated a major gene with a frequency q_s of .574 ± .065, codominant effects (μ_{ss} = −.909 ± .136; μ_{Ss} = .131 ± .164; and μ_{SS} = 1.288 ± 0.179), and a residual SD of 0.676 ± 0.052.

Combined Segregation and Linkage Analysis

The results of combined segregation and linkage analysis are shown in table 5. The first model fitted was a model assuming the cosegregation of a major gene and a marker in linkage disequilibrium (model 1). When compared with this model, the model assuming no major gene was strongly rejected (χ^2 = 153.83 with 4 df; $P < .001$). The hypothesis of gametic equilibrium (model 2) was also strongly rejected (χ^2 = 114.23 with 1 df; $P < .001$). The test of π_I to 1 suggested that the disequilibrium was complete, the allele I of the marker being always associated with the major gene allele s (model 3 vs. model 1) (χ^2 = 2.02 with 1 df; NS). However, there was not a complete identity between the two loci (model 4 vs. model 3) (χ^2 = 10.48 with 1 df; $P < .001$). Last, including a residual polygenic component (model 5) did not significantly improve the likelihood (χ^2 = 3.17 with 1 df; NS). It could be verified that model 4 was equivalent to model 1 of table 3, as mentioned in the Subjects and Methods section.

The best-fitting model (model 3) indicated a major

Table 4

Segregation Analysis: Hypotheses Testing Contribution of Unmeasured Major Gene to Variability of Plasma ACE Level

	Model 1	Model 2	Model 3
Major-gene frequency: q_s	.574	.562	.658
Transmission probabilities:			
τ_1	(1)[a]	1[b]	.673
τ_2	(.5)[a]	.415	(.673)[a]
τ_3	(0)[a]	.307	(.673)[a]
Major genotype-specific means:			
μ_{ss}	−.909	−.983	−.808
μ_{Ss}	.131	.127	.398
μ_{SS}	1.288	1.379	1.586
Residual SD	.676	.597	.627
Residual h^2	0[b]	(0)[a]	(0)[a]
Alternate model	0[c]	1	2
χ^2	37.27***	6.18 (NS)	34.70***
df	4	3	2

[a] Parameter fixed.
[b] Parameter estimated to a boundary value.
[c] Model 0 is without major-gene and polygenic component.
*** $P < .001$.

Table 5

Combined Segregation and Linkage Analysis: Hypotheses Testing Transmission of Major Gene in Linkage Disequilibrium with I/D Polymorphism

	Model 1	Model 2	Model 3	Model 4	Model 5
Frequency of marker allele I: f_I	.431	.431	.431	.431	.431
Frequency of major-gene allele s:					
Conditional on allele I, π_I	.950	.584	(1)[a]	(1)[a]	(1)[a]
Conditional on allele D, π_D	.212	(.584)[a]	.221	(0)[a]	.185
Major genotype-specific means:					
μ_{ss}	−.976	−.884	−.888	−.930	−.900
μ_{Ss}	.070	.175	.137	−.079	.086
μ_{SS}	1.094	1.234	1.066	.650	1.001
Residual SD	.708	.697	.745	.850	.765
Residual h^2	(0)[a]	(0)[a]	(0)[a]	(0)[a]	.187
Alternate model	0[b]	1	1	3	3
χ^2	153.83***	114.23***	2.02 (NS)	10.48***	3.17 (NS)
df	4	1	1	1	1

[a] Parameter fixed.
[b] Model 0 is without major gene effects.
** $P < 0.01$.
*** $P < .001$.

gene in complete linkage disequilibrium with the I/D polymorphism, with the two alleles s and S having codominant effects, and no residual heritability. Parameters estimates (mean ± SD) under this model were $f_I = .431 \pm .025$, $\pi_D = 0.221 \pm 0.064$, $\mu_{ss} = -0.888 \pm 0.079$; $\mu_{Ss} = 0.137 \pm 0.091$; $\mu_{SS} = 1.066 \pm 0.148$; and $\sigma = 0.745 \pm 0.037$. The frequency of the major allele s was derived from these parameters ($q_s = .557 \pm .041$). The major gene explained 44% of the variability of ACE level. Figure 1 shows the means of ACE level in the three genotypic classes defined by the major locus and by the marker locus, respectively. For the marker locus, the means were the mean values observed in the three genotypes and given in table 2. For the major locus, the means were derived from model 3 of table 5 and were retransformed in the units of origin. The means within the II genotype and the ss genotype were very close, reflecting the complete association of allele I with allele s.

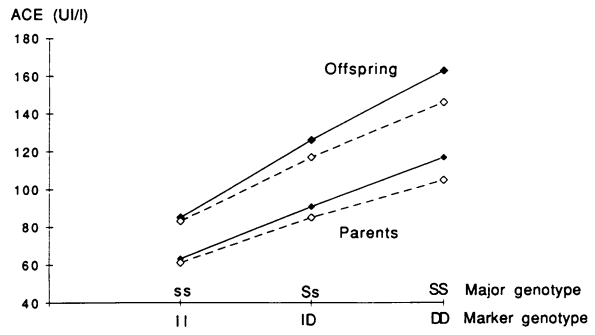

Figure 1 Means of plasma ACE level in the three genotype classes defined by the major gene and the I/D marker. For the marker locus, means are observed values. For the major locus, means are estimated from combined segregation and linkage analysis. ◆—◆ = Major gene; and ◇–◇ = marker.

Discussion

Families participating in this study were ascertained from the population at large and were selected on the basis of absence of (*a*) acute or chronic pathology and (*b*) treatment likely to modify the phenotype. Although the recruitment was based on volunteering, which might introduce a selection bias, the ACE levels measured in this sample can be considered as reflecting the distribution of this parameter in healthy individuals.

The ACE levels were slightly higher in men than in women, as generally has been reported elsewhere (Cambien et al. 1988; Bénéteau-Burnat et al. 1990). In offspring, the ACE activity gradually increases with age, until puberty, and then it decreases during adolescence, until adulthood (Bénéteau-Burnat et al. 1990). Although similar trends were observed in the present study, age accounted for only 10% of the variability

of ACE levels in offspring. The higher interindividual variability in offspring than in adults may be related to hormonal regulations activated during puberty, and it is likely that the adjustment for age only partly controlled for these variations.

The present study constituted a further step in the program initially designed to investigate the role of genetic factors in the determination of plasma ACE level. A first step was to explore the familial similarity in plasma ACE level in a sample of 87 healthy nuclear families also recruited in the Center for Preventive Medicine in Nancy (Cambien et al. 1988). The Nancy study demonstrated that the familial resemblance of ACE level could be almost entirely explained by the transmission of a major gene having codominant effects and contributing to a large part of the variability of the trait. However, segregation analysis provided no information on the particular region of the genome involved.

To test the hypothesis that an allelic variant of the ACE gene is responsible for this gene effect, an association study was designed to examine the relationship between circulating ACE levels and polymorphisms of the ACE gene in a sample of 80 healthy unrelated individuals (Rigat et al. 1990). An I/D polymorphism was detected, which accounted for 47% of the ACE phenotypic variance in the sample studied, a proportion somewhat larger than the 30% estimated in adults of the first study. These findings had to be confirmed by a new family study, which would also help to determine whether the I/D polymorphism was the variant responsible for the major-gene effects detected in the segregation analysis or was only a marker in linkage disequilibrium with this variant.

The characteristics of the families included in the present study were similar to those of the first family study, and the conclusions of the previous segregation analysis were confirmed. On the other hand, the measured genotype approach allowed us to demonstrate the association of the I/D polymorphism with the ACE level within families, reinforcing the findings of the association study including unrelated individuals. This polymorphism accounted for 28% of the total variance, a proportion lower than the 44% attributed to the putative major gene. Moreover, after adjustment for the polymorphism effects, significant heritability persisted, indicating that the segregation of the polymorphism could not entirely explain the familial resemblance of ACE level.

Finally, the results of the combined segregation and linkage analysis provided evidence that the major-gene effect detected in the segregation analysis was actually due to a variant of the ACE gene, a variant in strong linkage disequilibrium with the I/D polymorphism. This variant appeared very common, with allele frequencies of .56 and .44, making it difficult to interpret the effects as "protective" or "deleterious"; rather, it may be considered as a polymorphism affecting the variability of the ACE level, without reference to a normal level. The magnitude of the major-gene effect was equal to 1.3 residual SDs. As initial variances were higher in offspring than in parents, the ACE level was standardized separately in parents and offspring. Raw effects are then higher in offspring than in parents, indicating a possible genotype × age interaction, which was also suggested by the results of the analysis of variance, although this interaction did not reach statistical significance. Nevertheless, the higher variance within I/D genotypes (table 2) in offspring than in parents indicates that other factors contribute to the greater variability of this trait in childhood.

The results of these analyses strongly reinforce the hypothesis that the ACE gene is implicated in the control of the circulating ACE level and suggest that the I/D polymorphism is not the variant itself but, more likely, is a neutral marker. However, because of both the disequilibrium existing between the two loci (the marker allele I always being associated with the major allele s) and the closeness of allele frequencies, this marker appears to be very informative, with a sensitivity of 1 and a specificity of .77 (or the opposite, depending on which allele is considered "deleterious").

The considered phenotype in the present study is the concentration of circulating ACE, also called the "soluble" form of ACE. This latter form does not contain the carboxy-terminal end of the membrane-bound enzyme, which contains the anchoring peptide (Wei et al. 1991). It is not known yet whether plasma ACE totally derives from the bound form of the endothelial cells by a proteolytic process, as demonstrated in Chinese hamster ovary cells (Wei et al. 1991), or whether another ACE mRNA encodes for the soluble form. Two alternative hypotheses, then, might explain how the variant identified here affects plasma ACE levels. As a first hypothesis, the low level of ACE would be an indirect consequence of a low expression of the ACE gene. According to a second hypothesis, the plasma level would be separately modified by a mutation that might either alter the proteolytic cleavage of ACE or decrease the expression of the mRNA for the soluble form. Molecular techniques will be required to detect differences in the sequence of the ACE gene

between individuals with the II genotype and the DD genotype.

The identification of this variant may have several implications. By determining the genotype of a given individual, it would be possible to reduce substantially the size of the reference interval with which his or her ACE level should be compared. In particular, this should improve the diagnosis and the follow-up of granulomatous diseases, such as sarcoidosis, which are characterized by an elevation of the circulating ACE level (Lieberman 1975). Because of the key role that ACE plays in the renin-angiotensin system, the ACE gene is a candidate gene in primary hypertension. In recent linkage studies in genetically hypertensive rats, one locus associated with blood pressure regulation has been localized within a region containing the ACE gene (Hilbert et al. 1991; Jacob et al. 1991). This region forms part of a conserved linkage group, both on chromosome 10 in the rat and on human chromosome 17q23, which is where the human ACE gene is mapped (Mattei et al. 1989). In our study excluding hypertensive individuals, no association was found between the I/D polymorphism and blood pressure, but a weak correlation was found between blood pressure and ACE level in children. Further studies in hypertensive families will be necessary to test the implication of the ACE gene in human essential hypertension.

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References

Alhenc-Gelas F, Richard J, Courbon D, Warnet JM, Corvol P (1991) Distribution of plasma angiotensin I-converting enzyme levels in healthy men: relationship to environmental and hormonal parameters. J Lab Clin Med 117:33–39

Bénéteau B, Baudin B, Morgant C, Giboudeau J, Baumann FC (1986) Automated kinetic assay of angiotensin-converting enzyme in serum. Clin Chem 32:884–886

Bénéteau-Burnat B, Baudin B, Morgant G, Baumann FC, Giboudeau J (1990) Serum angiotensin-converting enzyme in healthy and sarcoidotic children: comparison with the reference interval for adults. Clin Chem 36:344–346

Boerwinkle E, Chakraborty R, Sing CF (1986) The use of measured genotype information in the analysis of quantitative phenotypes in man. Ann Hum Genet 50:181–194

Bonney GE, Lathrop GM, Lalouel J-M (1988) Combined linkage and segregation analysis using regressive models. Am J Hum Genet 43:29–37

Cambien F, Alhenc-Gelas F, Herbeth B, Andre JL, Rakotovao R, Gonzales MF, Allegrini J, et al (1988) Familial resemblance of plasma angiotensin-converting enzyme level: the Nancy study. Am J Hum Genet 43:774–780

Demenais F, Lathrop M, Lalouel JM (1986) Robustness and power of the unified model in the analysis of quantitative measurements. Am J Hum Genet 38:228–234

Deschamps JP (1987) Experience in France with periodic monitoring of family health. In: Harris EK, Yasaka T (eds) Maintaining a healthy state within the individual. Elsevier Science, New York, pp 3–8

Donner A, Koval JJ (1981) Multivariate analysis of family data. Am J Epidemiol 114:149–154

Dux S, Aron N, Boner G, Carmel A, Taron A, Rosenfeld JB (1984) Serum angiotensin converting enzyme activity in normal adults and patients with different types of hypertension. Isr J Med Sci 20:1138–1141

Elston RC, Stewart J (1971) A general model for the genetic analysis of pedigree data. Hum Hered 21:523–542

Erdös E, Skidgel RA (1987) The angiotensin I-converting enzyme. Lab Invest 56:345–348

Hasstedt SJ (1989) Pedigree analysis package. Department of Human Genetics, University of Utah, Salt Lake City

Hilbert P, Lindpaintner K, Beckmann JS, Serikawa T, Soubrier F, Dubay C, Cartwright P, et al (1991) Chromosomal mapping of two genetic loci associated with blood-pressure in hereditary hypertensive rats. Nature 353:521–529

Hubert C, Houot AM, Corvol P, Soubrier F (1991) Structure of the angiotensin I-converting enzyme gene: two alternate promoters correspond to evolutionary steps of a duplicated gene. J Biol Chem 266:15377–15383

Jacob HJ, Lindpaintner K, Lincoln SE, Kusumi K, Bunker RK, Mao YP, Ganten D, et al (1991) Genetic mapping of a gene causing hypertension in the stroke-prone spontaneously hypertensive rat. Cell 67:213–214

Lalouel JM, Rao DC, Morton NE, Elston RC (1983) A unified model for complex segregation analysis. Am J Hum Genet 35:816–826

Lieberman J (1975) Elevation of serum angiotensin converting enzyme level in sarcoidosis. Am J Med 59:365–372

Marcadet A, O'Connell P, Cohen D (1986) Standardized Southern blot workshop technique. In: Dupont B (ed) Histocompatibility testing, vol 1. Springer, New York, pp 553–560

Mattei MG, Hubert C, Alhenc-Gelas F, Roeckel N, Corvol P, Soubrier F (1989) Angiotensin I converting enzyme gene is on chromosome 17. Cytogenet Cell Genet 51:1041

Morton NE, MacLean CJ (1974) Analysis of family resemblance. III. Complex segregation analysis of quantitative traits. Am J Hum Genet 26:489–503

Rigat B, Hubert C, Alhenc-Gelas F, Cambien F, Corvol P, Soubrier F (1990) An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels. J Clin Invest 86: 1343–1346

Saiki RK, Gelfand DH, Stoffel S, Sharf SJ, Higuchi R, Horn GT, Mullis KB, et al (1988) Primer-directed enzymatic amplification of DNA with a thermostable DNA polymerase. Science 239:487–491

Wei L, Alhenc-Gelas F, Soubrier F, Michaud A, Corvol P, Clauser E (1991) Expression and characterization of recombinant human angiotensin I-converting enzyme: evidence for a C-terminal transmembrane anchor and for a proteolytic processing of the secreted recombinant and plasma enzymes. J Biol Chem 266:5540–5546